

WHAT IS CLAIMED:

1. A method for screening chemically modified mutant enzymes for amidase and/or esterase activity comprising:  
5 providing a chemically modified mutant enzyme with one or more amino acid residues from an enzyme being replaced by cysteine residues, wherein at least some of the cysteine residues are modified by replacing thiol hydrogen in the cysteine residues with a thiol side chain;  
contacting the chemically modified mutant enzyme with a substrate  
10 for an amidase and/or a substrate for an esterase; and  
determining whether the chemically modified mutant enzyme exhibits amidase and/or esterase activity.

2. A method according to claim 1, wherein the chemically modified mutant enzyme is screened for amidase activity.

15 3. A method according to claim 1, wherein the chemically modified mutant enzyme is screened for esterase activity.

4. A method according to claim 1, wherein said providing a chemically modified mutant enzyme comprises:

20 providing cysteine mutants of an enzyme, wherein one or more amino acid residues in the enzyme are replaced by cysteine residues;  
providing methanthiosulfonate reagents; and  
combining the cysteine mutants of an enzyme, the methanethiosulfonate reagents, and a buffer solution, wherein the cysteine residues are modified by replacing thiol hydrogen in the cysteine residue with a  
25 thiol side chain to form the chemically modified mutant enzyme.

5. A method according to claim 1 further comprising:  
providing a plurality of chemically modified mutant enzymes with one or more amino acid residues from enzymes being replaced by cysteine residues, wherein the cysteine residues are modified by replacing thiol hydrogen  
30 in at least some of the cysteine residues with a thiol side chain;

00038940-082401  
T07280-04682660

contacting the plurality of chemically modified mutant enzymes with a substrate for an amidase and/or a substrate for an esterase; and determining whether the plurality of chemically modified mutant enzymes exhibit amidase and/or esterase activity.

5                    6.        A method according to claim 1, wherein the ratio of chemically modified mutant enzyme to substrate is from about 1 M:10 M to about 1 M:10<sup>8</sup> M.

10                   7.        A method according to claim 1, wherein the ratio of chemically modified mutant enzyme to substrate is from about 1 M:10 M to about 1 M:10<sup>10</sup> M.

8.        A method according to claim 1, wherein the enzyme is a protease.

9.        A method according to claim 8, wherein the protease is a *Bacillus lentus* subtilisin.

15                   10.       A method according to claim 1, wherein the amino acid replaced with a cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.

11.       A method according to claim 1, wherein the amino acid replaced with a cysteine is in a subsite of the enzyme.

20                   12.       A method according to claim 11, wherein the subsite is selected from the group consisting of S<sub>1</sub>, S<sub>1</sub>', and S<sub>2</sub>.

13.       A method according to claim 1, wherein the thiol side chain is selected from the group consisting of -SCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, -SCH<sub>2</sub>(*p*-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>), -SCH<sub>2</sub>(*p*-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>), -SCH<sub>2</sub>(*p*-COOH-C<sub>6</sub>H<sub>4</sub>), -SCH<sub>2</sub>C<sub>6</sub>F<sub>5</sub>, -SCH<sub>2</sub>(*p*-CF<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>), and -SCH<sub>2</sub>(2,4-diNO<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>).

14.       A chemically modified mutant enzyme with one or more amino acid residues from an enzyme being replaced by cysteine residues, wherein at least some

099338940-082404  
FOI280 0468660

of the cysteine residues are modified by replacing thiol hydrogen in the cysteine residue with a thiol side chain, wherein the thiol side chain is selected from the group consisting of  $-\text{SCH}_2(p\text{-CH}_3\text{-C}_6\text{H}_4)$ ,  $-\text{SCH}_2(p\text{-OCH}_3\text{-C}_6\text{H}_4)$ ,  $-\text{SCH}_2(p\text{-CF}_3\text{-C}_6\text{H}_4)$ , and  $-\text{SCH}_2(2,4\text{-diNO}_2\text{-C}_6\text{H}_3)$ .

5                    15.    A chemically modified mutant enzyme according to claim 14, wherein the enzyme is a protease.

                    16.    A chemically modified mutant enzyme according to claim 15, wherein the protease is a *Bacillus lentus* subtilisin.

10                   17.    A chemically modified mutant enzyme according to claim 14, wherein the amino acid replaced with a cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.

15                   18.    A chemically modified mutant enzyme according to claim 14, wherein the amino acid replaced with a cysteine is in a subsite of the enzyme.

                    19.    A chemically modified mutant enzyme according to claim 18, wherein the subsite is selected from the group consisting of  $S_1$ ,  $S_1'$ , and  $S_2$ .

20                   20.    A chemically modified mutant enzyme according to claim 14, wherein the thiol side chain is  $-\text{SCH}_2(p\text{-CH}_3\text{-C}_6\text{H}_4)$ .

                    21.    A chemically modified mutant enzyme according to claim 14, wherein the thiol side chain is  $-\text{SCH}_2(p\text{-OCH}_3\text{-C}_6\text{H}_4)$ .

25                   22.    A chemically modified mutant enzyme according to claim 14, wherein the thiol side chain is  $-\text{SCH}_2(p\text{-CF}_3\text{-C}_6\text{H}_4)$ .

                    23.    A chemically modified mutant enzyme according to claim 14, wherein the thiol side chain is  $-\text{SCH}_2(2,4\text{-diNO}_2\text{-C}_6\text{H}_3)$ .

30

00938940 082401

providing an enzyme wherein one or more amino acids have been replaced with cysteine residues and

5 replacing thiol hydrogen in at least some of the cysteine residues with a thiol side chain to form the chemically modified mutant enzyme, wherein the thiol side chain is selected from the group consisting of  $-\text{SCH}_2(p\text{-CH}_3\text{-C}_6\text{H}_4)$ ,  $-\text{SCH}_2(p\text{-OCH}_3\text{-C}_6\text{H}_4)$ ,  $-\text{SCH}_2(p\text{-CF}_3\text{-C}_6\text{H}_4)$ , and  $-\text{SCH}_2(2,4\text{-diNO}_2\text{-C}_6\text{H}_3)$ .

25. A method according to claim 24, wherein the enzyme is a protease.

10

26. A method according to claim 25, wherein the protease is a *Bacillus lentus* subtilisin.

27. A method according to claim 24, wherein the amino acid replaced  
15 with a cysteine is an amino acid selected from the group consisting of asparagine, leucine,  
and serine.

28. A method according to claim 24, wherein the amino acid replaced with a cysteine is in a subsite of the enzyme.

20

29. A method according to claim 28, wherein the subsite is selected from the group consisting of S<sub>1</sub>, S<sub>1</sub>', and S<sub>2</sub>.

30. A method according to claim 24, wherein the thiol side chain is  
25  $-\text{SCH}_2(p\text{-CH}_3\text{-C}_6\text{H}_4)$ .

31. A method according to claim 24, wherein the thiol side chain is  $-\text{SCH}_2(p\text{-OCH}_3\text{-C}_6\text{H}_4)$ .

32. A method according to claim 24, wherein the thiol side chain is

30  $-\text{SCH}_2(p\text{-CF}_3\text{-C}_6\text{H}_4)$ .

33. A method according to claim 24, wherein the thiol side chain is

-SCH<sub>2</sub>(2,4-diNO<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>).

34. A detergent additive comprising the chemically modified mutant enzyme of claim 14.

5 35. A feed additive comprising the chemically modified mutant enzyme of claim 14.

36. A method of treating a textile comprising:  
providing a chemically modified mutant enzyme according to  
claim 14 and

10 contacting the chemically modified mutant enzyme with a textile  
under conditions effective to produce a textile resistant to enzyme-sensitive stains.

00938940 082401  
T04280 0468E660